New DNA-sensor based on thiacalix[4]arene-modified polydiacetylene particles

A. M. Valiyakhmetova,[a] E. D. Sultanova,[a,b] V. A. Burilov,[a]★ S. E. Solovieva,[a,b] and I. S. Antipin[a,b]

[a]*Kazan (Volga Region) Federal University,*
18 ul. Kremlevskaya, 420008 Kazan, Russian Federation.
E-mail: ultrav@bk.ru
[b]*A. E. Arbuzov Institute of Organic and Physical Chemistry, Kazan Scientific Center of the Russian Academy of Sciences,*
8 ul. Akad. Arbuzova, 420008 Kazan, Russian Federation

New *p-tert*-butyl thiacalix[4]arene derivative in the 1,3-*alternate* stereoisomeric form containing two diethylenetriamine groups and pentacosa-10,12-diynoic moieties on the opposide sides of macrocyclic cavity was synthesized using the copper(I)-catalyzed azide—alkyne cycloaddition. According to the dynamic and electrophoretic light scattering data, the synthesized macrocycle forms submicron particles with the sizes 200 nm and ζ-potential equal to 43 mV. The critical aggregation concentration of the macrocycle was 0.019 mmol L^{-1}. The obtained macrocycle intercalates into calf thymus DNA (CT DNA) to form a lipoplex. Using ethidium bromide as a fluorescent probe intercalation of obtained macrocycle into CT DNA with following formation of a lipoplex with the ζ-potential equal to −30 mV was found. The macrocycle was used for the synthesis of mixed polydiacetylene particles with *N*-(2-aminoethyl)pentacosa-10,12-diynamide (PCDA) as a base lipid. The highest degree of polymerization is achieved in the system with the macrocycle to PCDA ratio equal to 1 : 4. Macrocycle embedding into the polydiacetylene particles significantly increases their colorimetric response to CT DNA. The response to CT DNA as a change in the color of a solution of particles from blue to red is seen by naked eye at the CT DNA concentration starting from 20 μmol L^{-1}, which makes the obtained particles promising for bioanalytical application.

Key words: thiacalix[4]arene, polydiacetylenes, DNA-sensors, colorimetric sensors.

DNA assays are widely used in clinical practice to detect and study diseases caused by genetic defects.[1,2] Among the methods used for DNA detection, DNA electrophoresis in agarose gel is most popular, where two-chain DNA is visualized due to the addition of the intercalating dye (ethidium bromide).[3] Although this method is precise and commonly acknowledged, it requires special equipment and the corresponding professional skill of researchers, which makes it inappropriate for express diagnostics. In addition to gel electrophoresis, DNA determination can be performed using electrochemical analysis,[4] optical methods,[5] and mass sensitive methods,[6] which also demands special equipment. Thus, in spite of availability of diverse methods for DNA determination, the development of express methods still remains to be an urgent task. Therefore, colorimetric sensors based on polydiacetylenes (PDA)[7] with a unique conjugated structure sensitive to pH, temperature, mechanical action, and binding with analytes of various nature. The response of PDA to various effects is a change in the geometry of orbital overlapping between the ene—yne fragments of the conjugated polymer chain, which is visualized as a change in the color (usually from blue to red).[8] The nature of functional groups arranged on the polymer surface is decisive for the development of colorimetric sensors, since they determine selectivity and efficiency of interaction between PDA and analyte. Positively charged PDA particles with amino groups on the surface were successfully used for the determination of nucleic acids[9] and for the transfection of cells as lipoplexes with plasmid DNA.[10] We have previously prepared amphiphilic derivatives of *p-tert*-butylthiacalix-[4]arene (T[4]CA) containing diethylenetriamine moieties and found that they are efficiently intercalated in DNA to form compact lipoplexes.[11] Therefore, macrocycle conjugation to the polydiacetylene matrix seems to be promising for both the production of colorimetric biosensors and tasks of genetic engineering.

Thus, in this work, we discuss the synthesis of the T[4]CA derivative containing diethylenetriamine and 10,12-pentacosadiynamide moieties in the *1,3-alternate* configuration and the preparation of the related PDA particles for the colorimetric determination of DNA.

Results and Discussion

Synthesis of T[4]CA monomer with diethylenetriamine and diacetylene moieties. For the formation of polydiacetylenic particles, amphiphilic diacetylene monomers

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 5, pp. 1067—1074, May, 2019.
1066-5285/19/6805-1067

capable of self-organizing in aqueous solutions with the formation of vesicular structures are used. In such vesicular structures the mutual arrangement of the diacetylene fragments, which is necessary for the subsequent topochemical photopolymerization is achieved. Earlier we have proposed the universal approach to the synthesis of a broad series of amphiphilic macrocycles, being the consecutive modification of the T[4]CA platform by alkyl and azide fragments with the possibility of embedding any polar substituents by the copper-catalyzed azide—alkyne cycloaddition,[12,13] which was also used for the synthesis of the derivatives with diacetylene fragments.[14,15]

Macrocycle **1** in the *1,3-alternate* stereoisomeric form was synthesized using an earlier developed procedure[15] including the bis(alkylation) of T[4]CA by azidopropanol under Mitsunobu reaction conditions followed by bis(alkylation) by *N*-(3-hydroxypropyl)phthalimide, hydrazinolysis, and final acylation of the obtained diamine by pentacosa-10,12-diynic acid chloride. The reaction of diazide **1** with *N*,*N*-bis[2-(*tert*-butoxycarbonylamino) ethyl]propargylamine was carried out in toluene using the CuI—NEt_3 catalytic system for 12 h (Scheme 1). Product **2** was isolated in a yield of 85%. The structure and composition of the product were established by a complex of physicochemical methods. The ^{1}H NMR spectrum of product **2** ($CDCl_3$, 25 °C) exhibits a singlet of protons of the triazole ring at 7.56 ppm, a series of signals of methylenic protons of the ethylenediamine fragments as broadened singlets at 2.58, 3.22, and 3.83 ppm, and the signal of protons of the *tert*-butyl groups as a singlet at 1.44 ppm. The MALDI mass spectrum contains peaks $[M + H]^+$ with *m/z* 2397 and $[M + Na]^+$ with *m/z* 2419. The removal of the protective Boc groups was conducted using hydrogen chloride in dioxane, and the target amine **3** was obtained in a yield of 93% as dihydrochloride. The signal broadening related to the affinity of the compound to aggregation and the absence of signals from the protective Boc groups are observed in the ^{1}H NMR spectrum of product **3** (DMSO-d_6, 25 °C). The MALDI mass spectrum exhibits peak $[M + H - 2HCl]^+$ with *m/z* 1998.

Study of aggregation of macrocycle 3 and interaction with CT DNA. Macrocycle **3** with the amine headgroups turned out to be moderately soluble in a 25 m*M* buffer solution of tris(hydroxymethyl)aminomethane (Tris, pH 7.3). According to the data of dynamic (DLS) and electrophoretic light scattering (ELS) (Table 1), macrocycle **3** forms submicronic particles with the sizes 200 nm and ζ-potential equal to 43 mV. The critical aggregation concentration (CAC) of macrocycle **3** was determined fluorimetrically using pyrene as a fluorescent probe by measuring the ratio of intensities of the first (373 nm) and third (383 nm) peaks in the emission spectrum of pyrene, which varies upon pyrene solubilization to the hydrophobic layer of micelles or vesicles and is equal to 0.019 mmol L^{-1}.[16]

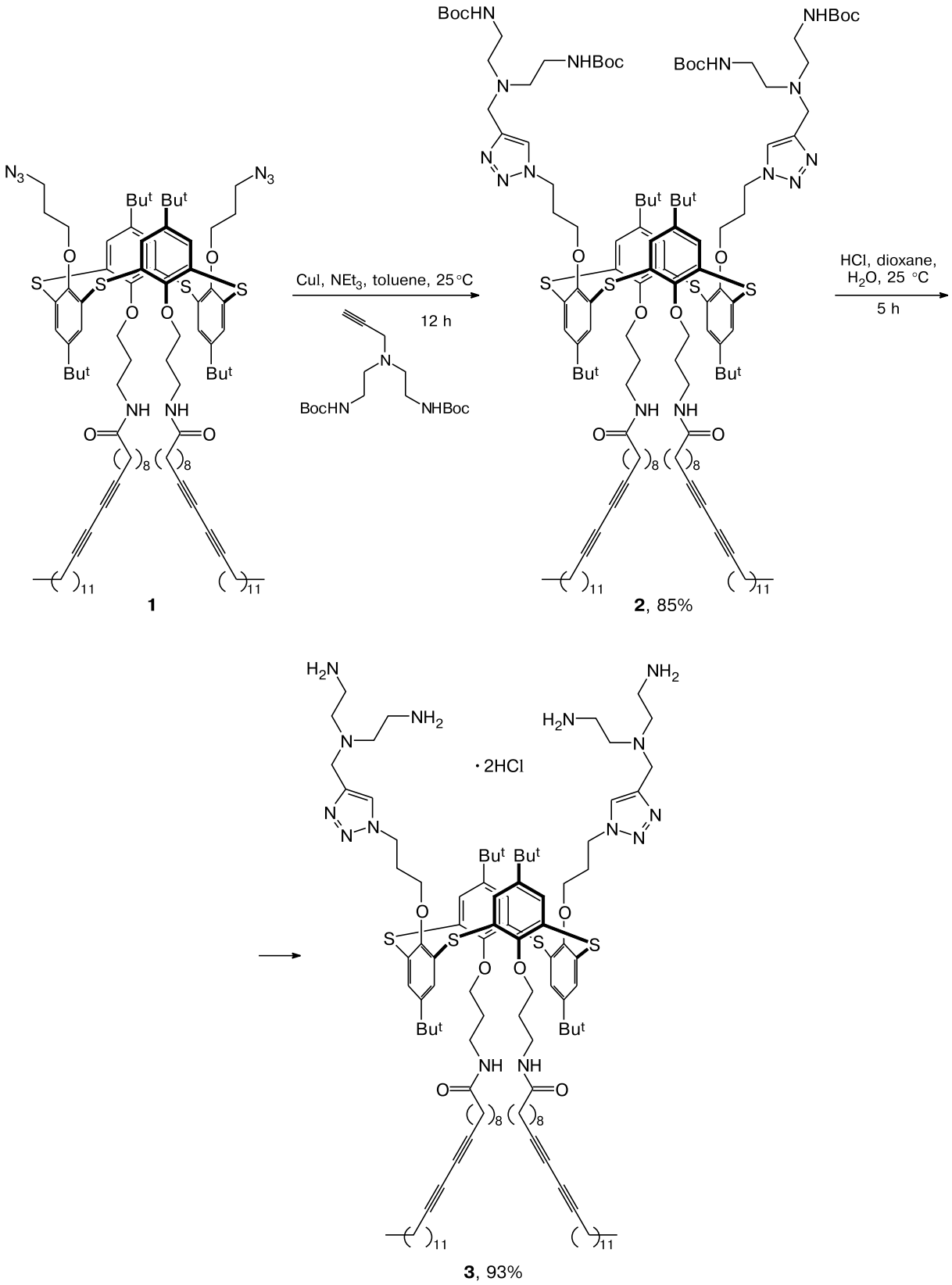
It is known that calf thymus DNA (CT DNA) is one of the most popular model nucleic acids, which is widely used for the study of interactions with the calixarene derivatives.[11,17] The fluorescence method based on the competitive displacement of the intercalating agent for DNA, ethidium bromide (EB) dye, was used to study binding of **3** with DNA. It is known that EB intercalates into DNA due to stacking with base pairs[18] followed by the hypsofluoric shift of the EB emission maximum accompanied by a substantial hyperfluoric effect. A portion of EB is displaced upon the interaction with competitive intercalating molecules, resulting in quenching of the EB emission. It was found that the addition of macrocycle **3** to a CT DNA—EB binary system leads to a substantial quenching of EB fluorescence accompanied by the bathofluoric shift of the emission maximum (from 601 nm to

Table 1. DLS and ELS data for the systems **3** + *N*-(2-aminoethyl)pentacosa-10,12-diynamide (PCDA) in the presence/absence of CT DNA*

System	Average hydrodynamic diameter/nm	Polydispersity index	ζ-potential/mV
3	190±73	0.391±0.001	43±03
3 + PCDA, 1 : 1**	2275±343	0.925±0.074	—***
3 + PCDA, 0.5 : 1**	1655±10; 841±36	0.526±0.018	54±9
3 + PCDA, 0.25 : 1**	76±2; 60±9	0.449±0.019	50±7
PCDA	712±55; 22±20	1.000±0.000	41±6
CT DNA	266±24	0.740±0.400	−36±2
CT DNA + **3**	220±16	0.420±0.002	−30±5
CT DNA + (**3** + PCDA, 1 : 1)**	495±19; 74 ±11	0.750±0.244	0
CT DNA + (**3** + PCDA, 0.5 : 1)**	—***	—***	—***
CT DNA + (**3** + PCDA, 0.25 : 1)**	719±28; 32±20	0.486±0.005	−5±5
CT DNA + PCDA**	1373±187; 266±65	0.468±0.034	0

* C_{PCDA} = 0—0.2 mmol L^{-1}, C_3 = 0—0.2 mmol L^{-1}, C_{DNA}(CT) = 0.09 mmol L^{-1} in Tris buffer (25 mmol L^{-1}).
** UV-polymerized system.
*** Failed to detect because of colloidal instability of the sample.

Scheme 1



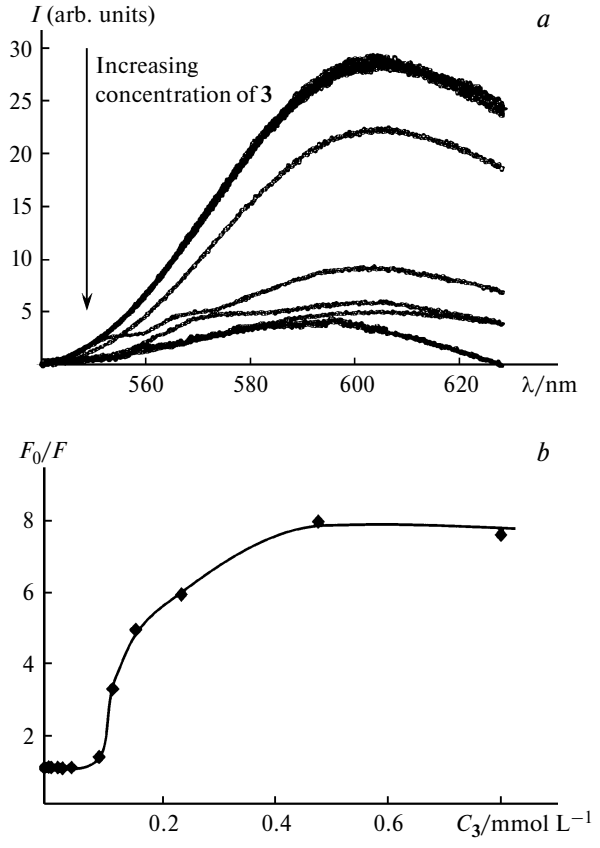


Fig. 1. (*a*) Fluorescence emission spectra of EB in the CT DNA—EB system in the presence of various amounts of **3** (0—0.81 mmol L^{-1}); (*b*) fluorescence titration in the Stern—Volmer coordinates at the emission maximum of EB— CT DNA (601 nm); C_{EB} = 8 μmol L^{-1}, C_{DNA}(CT) = 0.025 mmol L^{-1}; C_{Tris} = 25 mmol L^{-1}, pH 7.3.

589 nm), indicating the competitive intercalation of the macrocycle and displacement of EB from furrows of CT DNA (Fig. 1, *a*). An analysis of the dependence of the maximum of EB emission on the concentration of macrocycle **3** plotted in the Stern—Volmer coordinates shows that the complete displacement of EB from furrows of CT DNA occurs at a 20-fold excess of macrocycle **3** (Fig. 1, *b*). The most intense EB quenching starts at the concentration of **3** equal to 0.1 mmol L^{-1}, which is higher than the CAC of the macrocycle by five times. It is important that the dependence of luminescence quenching in the Stern—Volmer coordinates is nonlinear, indicating the mixed static and dynamic mechanisms of quenching of EB luminescence and making impossible the correct calculation of the quenching constant.[19]

According to the DLS and ELS data (see Table 1), the hydrodynamic diameter of the particles decreases to 220 nm after the addition of DNA to macrocycle **3**, and this is accompanied by surface recharging to −30 mV. This can be a result of covering of cationic particles of macrocycle **3** by the CT DNA molecule, which often occur when DNA interacts with cationic surfactants.[20] Thus, macrocycle **3** efficiently intercalates into CT DNA causing the displacement of EB to form a lipoplex, which has sizes of ~200 nm and thus is promising for use as a DNA-binding module in the composition with PDA vesicles.

Preparation of PDA vesicles doped with macrocycle 3 and study of their interaction with CT DNA. Cationic lipid PCDA (Scheme 2) known by its ability to form polydiacetylene liposomes in aqueous solutions under UV irradiation was chosen as a base lipid to prepare PDA vesicles.[21]

PDA vesicles consisting of both PCDA itself and containing macrocycle **3** in various amounts (Table 2) were prepared by the hydrophilization of the film followed by irradiation for 15 min at 254 nm in Tris buffer (25 m*M*, pH 7.3).

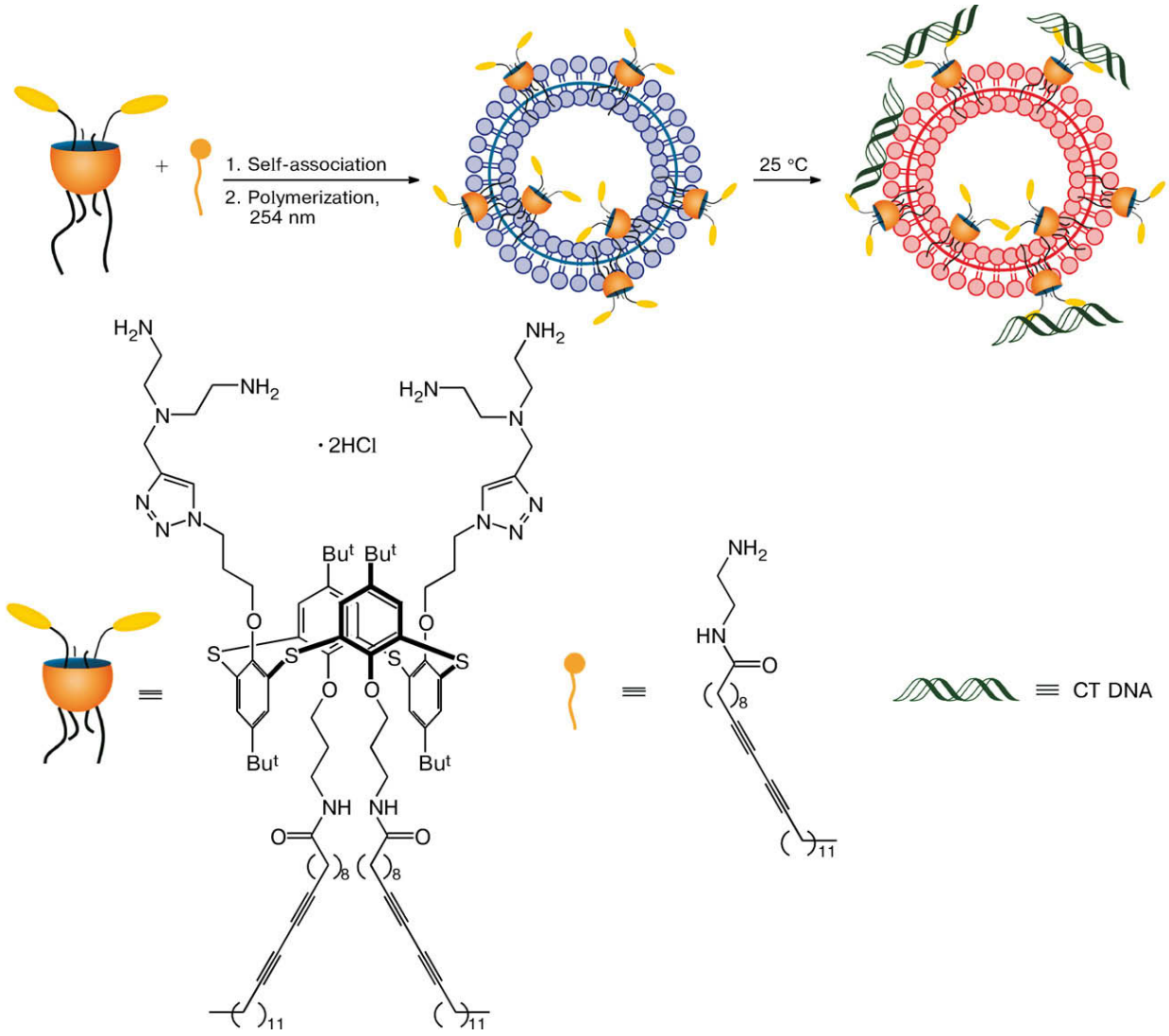
Colorless mixed vesicles were colored in diverse blue shades after irradiation, and the color did not change in the absence of PDA, indicating that macrocycle **3** cannot independently form extended conjugated PDA structures (Fig. 2, *a* and *b*). The sizes and charge of the obtained particles were studied by the DLS and ELS methods (see Table 1). According to the obtained data, PCDA form bimodal submicronic lamellar structures with high polydispersity index, and the addition of a small amount of **3** (1 : 4) results in a substantial decrease in the particle size and a decrease in the polydispersity index. It is characteristic that the maximum absorption at 640 nm is achieved at the **3** : PCDA ratio equal to 1 : 4, indicating the highest degree of polymerization of particles close to the polymerization of pure PCDA (Fig. 2, *a*, curves *1* and *2*). The further increase in the content of macrocycle **3** leads to an increase in the sizes and polydispersity index of PDA particles and also to a decrease in the degree of polymerization of PCDA (see Fig. 2, *a*). Thus, the addition of a small amount of calixarene results in the colloidal stabilization of particle and an insignificant decrease in the degree of polymerization of PCDA.

Taking into account the cationic nature of head groups and the demonstrated ability of macrocycle **3** to interact

Table 2. Concentrations of the reagents for the preparation of mixed lipid vesicles **3** + PCDA (total concentration 1.2 mmol L^{-1})

Ratio **3** : PCDA	C_3	C_{PCDA}
	mmol L^{-1}	
1 : 0	1.2	0
1 : 1	0.6	0.6
0.5 : 1	0.4	0.8
0.25 : 1	0.24	0.96
0 : 1	0	1.2

Scheme 2



Note. Scheme 2 is available in full color on the web page of the journal (http://www.link.springer.com/journal/11172).

with CT DNA, the vesicles (**3** + PCDA) were used as a colorimetric sensor for CT DNA determination (see Scheme 2). The colorimetric response (CR) was calculated by the equation

$$CR\ (\%) = [(PB_0 - PB_n)/PB_0] \cdot 100\%, \qquad (1)$$

where $PB = A_{blue}/(A_{blue} + A_{red})$; A_{blue} is the absorbance intensity at ~640 nm, A_{red} is the absorbance intensity at ~540 nm, and PB_0 and PB_n are the ratios of A_{blue} to A_{red} of the control sample in the absence and presence of DNA, respectively. It is found that macrocycle **3** embedding in PDA particles of PCDA leads to a substantial increase in the sensitivity toward CT DNA compared to the PDA particles of PCDA (Fig. 3, *a*). The colorimetric response of the system is significant and can be seen by naked eye at the CT DNA concentration equal to 20 μmol L^{-1}, and linearity of the colorimetric response is retained in the range of CT DNA concentrations 20—250 μmol L^{-1}.

According to the ELS data (see Table 1), the addition of CT DNA to PDA particles substantially decreases the electrokinetic potential (ζ-potential) making, in some cases, impossible correct estimation of the sizes and charge. Thus, the efficient interaction of anionic CT DNA with the cationic PDA particle results in the combined neutralization and sedimentation inducing a distortion of the geometry of the main polymer chain and a change in the color of the polymer. The addition of macrocycle **3**, which is affinic to CT DNA, results in an increase in the efficiency of interaction of the particles with CT DNA, which makes it possible to consider this system as promising for the colorimetric determination of CT DNA in the micromolar concentration range.

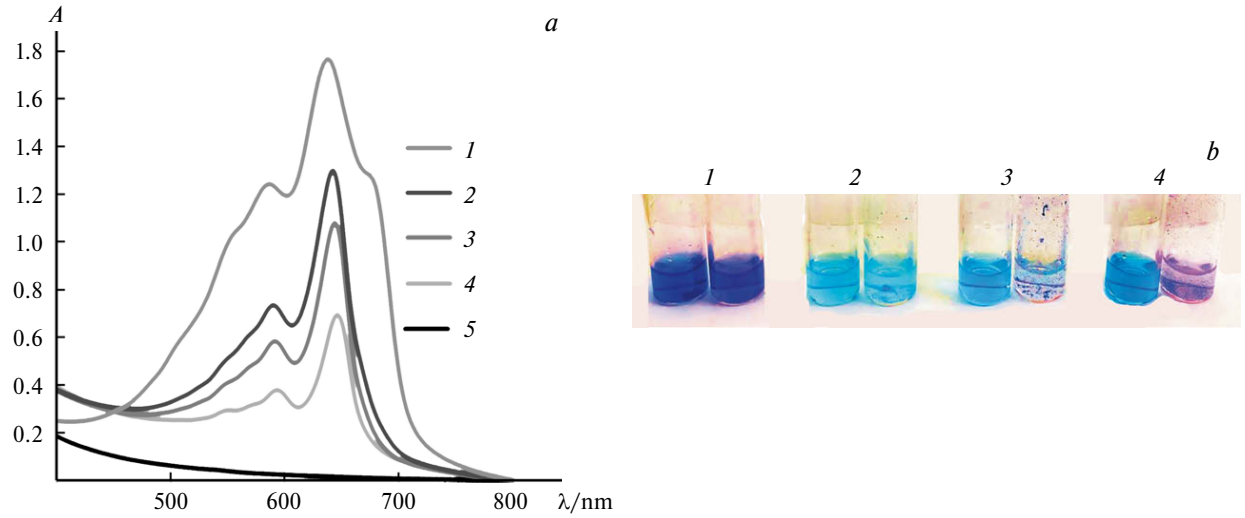


Fig. 2. (*a*) Electronic absorption spectra of polymer particles **3** + PCDA with the **3** : PCDA ratio equal to 0 : 1 (*1*), 0.25 : 1 (*2*), 0.5 : 1 (*3*), and 1 : 0 (*5*); (*b*) photographs of solutions containing **3** + PCDA with the **3** : PCDA ratio equal to 0 : 1 (*1*), 1 : 1 (*2*), 0.5 : 1 (*3*), and 0.25 : 1 (*4*), without CT DNA (at the left) and in the presence of 0.089 mmol L^{-1} CT DNA (at the right); C_{PCDA} = 0.2 mmol L^{-1}, C_3 = 0—0.2 mmol L^{-1} in Tris buffer (25 mmol L^{-1}, pH 7.3).

Note. Figures 2 and 3 are available in full color on the web page of the journal (http://www.link.springer.com/journal/11172).

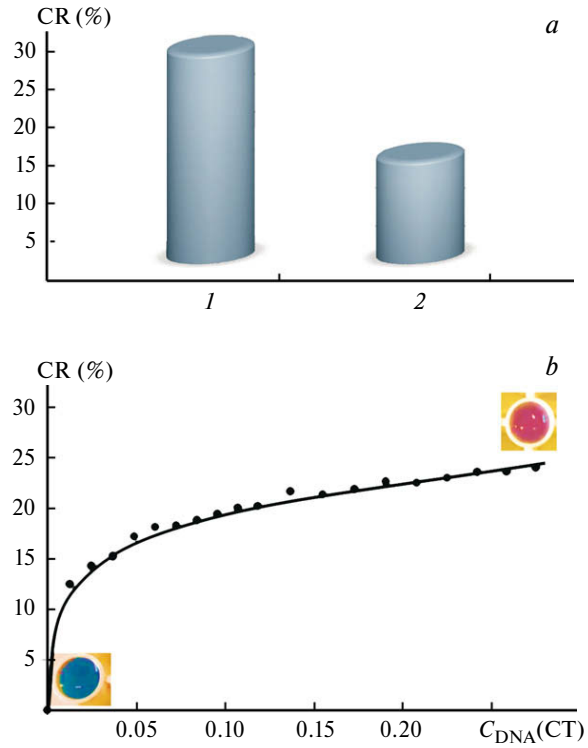


Fig. 3. (*a*) Colorimetric response (CR) of vesicles **3** + PCDA with the **3** : PCDA ratio equal to 0.25 : 1 (*1*) and 0 : 1 (*2*) in the presence of 0.09 mmol L^{-1} CT DNA; (*b*) CR of vesicles **3** + + PCDA with the **3** : PCDA ratio 0.25 : 1 *vs* concentration of CT DNA; C_{PCDA} = 0.2 mmol L^{-1}, C_3 = 0.05 mmol L^{-1}, C_{DNA}(CT) = 0—0.3 mmol L^{-1} in Tris buffer (25 mmol L^{-1}, pH 7.3).

To conclude, *p-tert*-butylthiacalix[4]arene derivative **3** containing two diethylenetriamine moieties at one side and pentacosa-10,12-diynamide fragments at another side of the macrocyclic cavity in the stereoisomeric form of 1,3-*alternate* has first been synthesized. It is found that the obtained macrocycle forms submicronic particles with the sizes 200 nm and the ζ-potential equal to 43 mV. The obtained macrocycle is shown to intercalate into CT DNA to form a lipoplex with the ζ-potential equal to –30 mV. Macrocycle **3** was used for the synthesis of mixed PDA particles with PCDA as a base lipid. It is established that macrocycle **3** embedding in the PDA particles leads to a substantial increase in their colorimetric response toward CT DNA, which allows one to determine CT DNA starting from 20 μmol L^{-1}, due to which the obtained particles become promising for bioanalytical application.

Experimental

Prior to use, solvents (reagent grade, analytical pure grade) were purified using known procedures.[22] Commercially available reagents from the Sigma-Aldrich and Alfa-Aesar catalogues were used. Macrocycle **1**,[15] *N*,*N*-bis[2-(*tert*-butoxycarbonylamino)-ethyl]propargylamine,[23] and *N*-(2-aminoethyl)-10,12-pentacosadiynamide[21] were synthesized according to previously published procedures.

Synthesis of 5,11,17,23-tetra-*tert*-butyl-25,27-bis{3-(pentacosa-10,12-diynamido)propoxy}-26,28-bis{3-(4-((bis(2-((*tert*-butyloxycarbonyl)amino)ethyl)methyl)-1*H*-1,2,3-triazol-1-yl)-propoxy}-2,8,14,20-tetrathiacalix[4]arene (2). Weighed samples of compound **1** (0.15 g, 0.09 mmol), *N*,*N*-bis[2-(*tert*-butoxycarbonylamino)ethyl]propargylamine (0.08 g, 0.22 mmol), triethylamine (0.6 mL, 4.37 mmol, 0.73 g mL^{-1}) and CuI (0.8 mg,

4.4 μmol) were dissolved in anhydrous toluene (4 mL). The reaction mixture was stirred for 12 h at room temperature in an inert atmosphere. After the end of the reaction, chloroform (30 mL) was added, and the organic phase was washed with an ammonia solution (3×15 mL) and water to pH 7 and dried over anhydrous $MgSO_4$. The solvent was removed on a rotary evaporator. The product was purified by column chromatography on silica gel (70—230 mesh, ethyl acetate as eluent), and a beige powder was obtained after solvent evaporation. The yield was 0.18 g (85%); R_f 0.56 (ethyl acetate as eluent). M.p. 168 °C. MALDI MS, m/z (I_{rel} (%)): 2397 $[M + H]^+$, 2419 $[M + Na]^+$. Found (%): C, 68.28; H, 9.15; N, 8.23; O, 9.26; S, 5.27. Calculated for $C_{136}H_{214}N_{14}O_{14}S_4$ (%): C, 68.13; H, 9.00; N, 8.18; O, 9.34; S, 5.35. IR, ν/cm^{-1}: 1701 (CO), 2855 (CH_2), 2927 (CH_3), 3308 (NH). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, δ): 0.88 (t, 6 H, CH_3, J = 6.7 Hz); 1.03—1.39 (m, 88 H, CH_2, CH_3); 1.44 (s, 36 H, $C(CH_3)_3$); 1.48—1.78 (m, 24 H, CH_2); 2.14 (t, 4 H, O=C—CH_2, J = 7.6 Hz); 2.24 (t, 8 H, C≡C—CH_2, J = 6.8 Hz); 2.58 (br.s, 8 H, CH_2N); 2.92—3.00 (m, 4 H, CONH—CH_2); 3.22 (br.s, 8 H, CH_2NHBoc); 3.80—3.86 (m, 8 H, NCH_2Trz*, CH_2Trz); 4.00 (t, 4 H, OCH_2, J = 6.8 Hz); 4.12 (t, 4 H, OCH_2, J = 7.2 Hz); 5.18 (br.s, 4 H, NH); 6.10 (t, 2 H, NH, J = 5.5 Hz); 7.27 (br.s, 4 H, H_{Ar}); 7.31 (br.s, 4 H, H_{Ar}); 7.56 (br.s, 2 H, TrzH). ^{13}C NMR (101 MHz, $CDCl_3$, 25 °C, δ): 14.28, 19.34, 22.83, 26.04, 28.49, 28.62, 29.00, 29.15, 29.24, 29.43, 29.49, 29.62, 29.77, 30.28, 31.23, 31.38, 32.05, 34.43, 36.77, 37.06, 38.34, 48.00, 53.05, 65.43, 66.45, 67.06, 122.97, 127.75, 128.20, 128.38, 146.19, 146.42, 156.44, 156.98, 173.16.

Synthesis of 5,11,17,23-tetra-*tert*-butyl-25,27-bis{3-(pentacosa-10,12-diynamido)propoxy}-26,28-bis{3-(4-((bis(2-(amino)-ethyl)amino)methyl)-1*H*-1,2,3-triazol-1-yl)propoxy}-2,8,14,20-tetrathiacalix[4]arene (3). A weighed sample of compound **2** (0.11 g, 0.05 mmol) was dissolved in dioxane (5 mL), and concentrated HCl (0.15 mL, 1.81 mmol, d = 1.189 g mL^{-1}) was added dropwise. The reaction mixture was stirred at room temperature for 5 h. To isolate the product, the reaction mixture was evaporated to dryness on a rotary evaporator to obtain a brown powder, which was then dried in a vacuum desiccator over P_2O_5. The yield was 0.096 g (93%). M.p. (with decomp.) 157 °C. MALDI MS, m/z (I_{rel} (%)): 1998 $[M + H - 2HCl]^+$. Found (%): C, 67.21; H, 8.99; N, 9.41; O, 4.68; S, 6.15. Calculated for $C_{116}H_{184}Cl_2N_{14}O_6S_4$ (%): C, 67.31; H, 8.96; N, 9.47; O, 4.64; S, 6.20. IR, ν/cm^{-1}: 1641 (CO), 2849 (CH_2) 2917 (CH_3), 3302 (NH). ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C, δ): 0.84 (t, 6 H, CH_3, J = 6.1 Hz); 1.05—1.70 (br.m, 112 H, CH_2, CH_3); 2.00—2.08 (br.m, 4 H, O=C—CH_2); 2.25 (br.t, 8 H, C≡C—CH_2); 2.75—3.27 (br.m, 20 H, CONH—CH_2, CH_2NHBoc); 3.70—3.92 (br.m, 8 H, NCH_2Trz, CH_2Trz); 4.24 (br.s, 4 H, OCH_2); 4.54 (br.s, 4 H, OCH_2); 7.32 (br.s, 8 H, H_{Ar}); 7.74 (br.s, 2 H, HTrz); 8.20—8.45 (br.m, 8 H, NH). ^{13}C NMR (101 MHz, DMSO-d_6, 25 °C, δ): 18.30, 27.70, 27.76, 28.16, 28.22, 28.42, 28.74, 28.89, 28.97, 29.04, 30.81, 30.88, 43.80, 46.90, 49.37, 62.80, 65.39, 77.75, 77.86, 127.48, 127.64, 145.79, 156.60, 172.05.

Synthesis of polymeric vesicles. Chloroform solutions of the reagents in the amounts presented in Table 1 were mixed, after which chloroform was removed with a nitrogen flow. The obtained films were hydrophilized at 65 °C in 3 mL of an aqueous solution of Tris buffer (pH 7.3, 25 mmol L^{-1}). The obtained mixture was kept at 4 °C for 15 h and then irradiated (λ = 254 nm) for 15 min in quartz cells.

Methods. NMR experiments were carried out on an Avance-400 Nanobay spectrometer (Bruker). Signals of residual protons of solvent $CDCl_3$ (δ_H 7.26) and DMSO-d_6 (δ_H 2.50) at 25 °C were used as internal standards.

IR spectra were recorded on a Bruker Vector-22 spectrometer in the wavenumber range 400—4000 cm^{-1} in KBr pellets.

TLC analysis was conducted on the Merck Silica gel 60 F_{254} plates, and spots were detected in UV light (254 nm).

MALDI mass spectra were obtained on a UltraFlex III TOF/TOF mass spectrometer in the linear mode using *p*-nitroaniline as a matrix. A Nd:YAG laser (λ = 355 nm) was used. Positively charged ions were detected.

Elemental analyses of the obtained compounds were performed using a PerkinElmer PE 2400 CHNS/O analyzer.

Melting points of the substances were determined on an OptiMelt MPA100 automated heating stage.

Experiments on dynamic and electrophoretic light scattering were carried out on a Zetasizer Nano ZS instrument (Malvern Instruments, USA) with the radiation source of a He—Ne lamp (power 4 mW, wavelength 633 nm, light scattering angle 173°). Data were processed using the DTS software (Dispersion Technology Software 5.00). Prior to measurements, solutions were filtered through the Millex HV filter (0.45 μm) to remove dust. At least three experiments in DTS-0012 disposable plastic cells (Sigma-Aldrich, USA) at 298 K (or in DTS-1070 capillary cells for ELS detection) were carried out for each system. Statistical data were processed using Student´s distribution, and the inaccuracy of particle size determination was <2%. Prior to measurements, the studied samples were subjected to the ultrasonic treatment for 30 min at 25 °C.

Fluorescence spectra were recorded in 10-mm quartz cells on a Fluorolog FL-221 spectrofluorimeter (HORIBA Jobin Yvon) in the range 350—430 nm at λ_{exc} = 335 nm with a slit of 2.5 nm for pyrene and in the range 540—670 nm at λ_{exc} = 500 nm with a slit of 2.5 nm for ethidium bromide. All studies were carried out in a Tris buffer solution (0.25 mmol L^{-1}, pH 7.3) at 25 °C.

Electronic absorption spectra were recorded in 10-mm quartz cells on a Shimadzu UV-2700 spectrophotometer in the range 400—800 nm. All studies were carried out in a Tris buffer solution (0.25 mmol L^{-1}, pH 7.3) at 25 °C.

To determine the CAC of macrocycle **3**, fluorescence titration was conducted by the method of molar ratios, where the pyrene concentration was maintained constant (0.001 mmol L^{-1}) and the concentration of macrocycle **3** was varied from 2 to $8.5 \cdot 10^{-6}$ mmol L^{-1}.

This work was financially supported by subsidy allocated to the Kazan Federal University for the state assignment in the sphere of scientific activities (4.1493.2017/4.6 and 4.5151.2017/6.7).

References

1. A. P. Turner, *Chem. Soc. Rev.*, 2013, **42**, 3184.
2. M. Demeunynck, C. Bailly, W. D. Wilson, *DNA and RNA Binders*, Eds M. Demeunynck, C. Bailly, W. D. Wilson, Wiley-VCH, Weinheim, 2003.
3. A. J. Jeffreys, V. Wilson, S. L. Thein, *Nature*, 1985, **314**, 67.

* Trz is triazole.

4. M. Labib, E. H. Sargent, S. O. Kelley, *Chem. Rev.*, 2016, **116**, 9001.
5. J. Homola, *Chem. Rev.*, 2008, **108**, 462.
6. J. Wang, *Nucleic Acid. Res.*, 2000, **28**, 3011.
7. X. Chen, G. Zhou, X. Peng, J. Yoon, *Chem. Soc. Rev.*, 2012, **41**, 4610.
8. M. A. Reppy, B. A. Pindzola, *Chem. Commun.*, 2007, **42**, 4317.
9. Y. K. Jung, T. W. Kim, J. Kim, J.-M. Kim, H. G. Park, *Adv. Funct. Mater.*, 2008, **18**, 701.
10. E. Morin, M. Nothisen, A. Wagner, J.-S. Remy, *Bioconjug. Chem.*, 2011, **22**, 1916.
11. R. R. Ibragimova, V. A. Burilov, A. R. Aimetdinov, D. A. Mironova, V. G. Evtugyn, Y. N. Osin, S. E. Solovieva, I. S. Antipin, *Macroheterocycles*, 2016, **9**, 433.
12. M. Meldal, C. W. Tornøe, *Chem. Rev.*, 2008, **108**, 2952.
13. S. E. Solovieva, V. A. Burilov, I. S. Antipin, *Macroheterocycles*, 2017, **10**, 134.
14. V. Burilov, A. Valiyakhmetova, D. Mironova, R. Safiullin, M. Kadirov, K. Ivshin, O. Kataeva, S. Solovieva, I. Antipin, *RSC Adv.*, 2016, **6**, 44873.
15. V. Burilov, A. Valiyakhmetova, D. Mironova, E. Sultanova, V. Evtugyn, Y. Osin, S. Katsyuba, T. Burganov, S. Solovieva, I. Antipin, *New J. Chem.*, 2018, **42**, 2942.
16. J. Aguiar, P. Carpena, J. A. Molina-Bolivar, C. Carnero Ruiz, *J. Coll. Interface Sci.*, 2003, **258**, 116.
17. L. S. Yakimova, D. B. Puplampu, G. A. Evtyugin, I. I. Stoikov, *Russ. Chem. Bull.*, 2017, **66**, 1515.
18. Q. Guo, M. Lu, L. A. Marky, N. R. Kallenbach, *Biochemistry*, 1992, **31**, 2451.
19. J. R. Lakowicz, *Principles of Fluorescence Spectroscopy. Quenching of Fluorescence*, Kluwer Academic, New York, 1999.
20. K. Liu, L. Zheng, C. Ma, R. Göstl, A. Herrmann, *Chem. Soc. Rev.*, 2017, **46**, 5147.
21. W. Thongmalai, T. Eaidkong, S. Ampornpun, R. Mungkarndee, G. Tumcharern, M. Sukwattanasinitta, S. Wacharasindhu, *J. Mater. Chem.*, 2011, **21**, 16391.
22. W. L. F. Armarego, C. L. L. Chai, *Purification of Laboratory Chemicals*, Eds W. L. F. Armarego, C. L. L. Chai, Elsevier, New York, 2009.
23. N. G. Brown, R. VanderLinden, E. R. Watson, R. Qiao, C. R. R. Grace, M. Yamaguchi, F. Weissmann, J. J. Frye, P. Dube, S. Ei Cho, M. L. Actis, P. Rodrigues, N. Fujii, J. M.

Received December 19, 2018;
in revised form March 12, 2019;
accepted March 29, 2019